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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and effect of substituent position on anti-inflammatory activity of 3-(halobenzyl)isocarbostyrils

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Abstract A series of new natural product skeleton based 3-(halobenzyl)isocarbostyrils (**2a**–**2i**), were designed and synthesized to examine the effect of position of different halide substituents on anti-inflammatory activity. The structure–activity relationship shows a significant influence of position of halide substituents on in vitro anti-inflammatory activity. 3-(*o*-halobenzyl)isocarbostyrils (**2a**, **2d,** and **2g**) showed the lowest activity most probably due to the closure of pharmacophore site by intramolecular hydrogen bond between halides and N–H of amide. In contrast, 3-(*p*-halobenzyl)isocarbostyrils **2c**, **2f**, and **2i** exhibited moderate to very good inflammatory activity. Compound **2c** (IC_{50} = 251.002 ± 2.910) was found to have comparable activity with the standard drug (Indomethacin, IC_{50} = 271.210 ± 2.127). To further understand the effect of position of halide substituents on 3-(halobenzyl)isocarbostyrils, computational POM was carried out. The study with constructive propositions may be helpful for the design of more potent analogs.

Keywords Isocarbostyrils · Synthesis · Anti-inflammatory · Petra/Osiris/Molinspiration (POM)

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Introduction

Isocarbostyril (isoquinolin-1(2*H*)-one) derivatives, the nitrogen analogs of isocoumarins (1H-2-benzopyran-1-ones) have gained considerable attention from synthetic and pharmaceutical chemistry community (Sugimoto *et al.*, 1995; Mokrosz *et al.*, 1997; Ouchi *et al.*, 2004; Guastavino *et al.*, 2006; Wang *et al.*, 2009; Wu *et al.*, 2010; Li and Chua, 2011; Tyagi *et al.*, 2012) because of their presence in natural products like thalifoline (Krane and Shamma, 1982), dorianine (Glushkov and Shklyaev, 2001), ruprechstyril (Pettit *et al.*, 2003), narciclasine (Rigby *et al.*, 2000), pancratistatin (Rigby *et al.*, 2000), lycoricidine (Hudlicky *et al.*, 2002), thalflavine (Aly *et al.*, 1989), marinamide and its methyl ester (Zhu and Lin, 2006) (Fig. 1) in addition to their role as versatile building blocks for the total synthesis of natural alkaloids (Heaney and Shuhaibar, 1995; Coelho *et al.*, 2003). Substituted isocarbostyrils exhibit various biological and medicinal applications such as antihypertensive activity (Guastavino *et al.*, 2006) and function as NK3 antagonists (Simonsen *et al.*, 2010), melatonin MT1 and MT2 receptor agonists (Mor *et al.*, 2010), JNK inhibitors (Asano *et al.*, 2008), and Rhokinase inhibitors (Lohn *et al.*, 2009). Some of them are also used as novel antitumor agents that inhibit eukaryotic protein synthesis at the ribosomal level (Kohlhagen *et al.*, 1998), inhibitors of topoisomerase I (Kohlhagen *et al.*, 1998) and orally active inhibitors of Lck kinase (Snow *et al.*, 2002), orally active 5-HT3 antagonists (Matsui *et al.*, 1992), and thymidylate synthase (TS) inhibitors (Li *et al.*, 1991), or for the treatment of stomach tumors and diseases of human brain cells (Glushkov and Shklyaev, 2001).

Inflammation is a defensive biological response of vascular tissues to harmful stimuli for physiological adaptations to limit tissue damage and initiate the healing process

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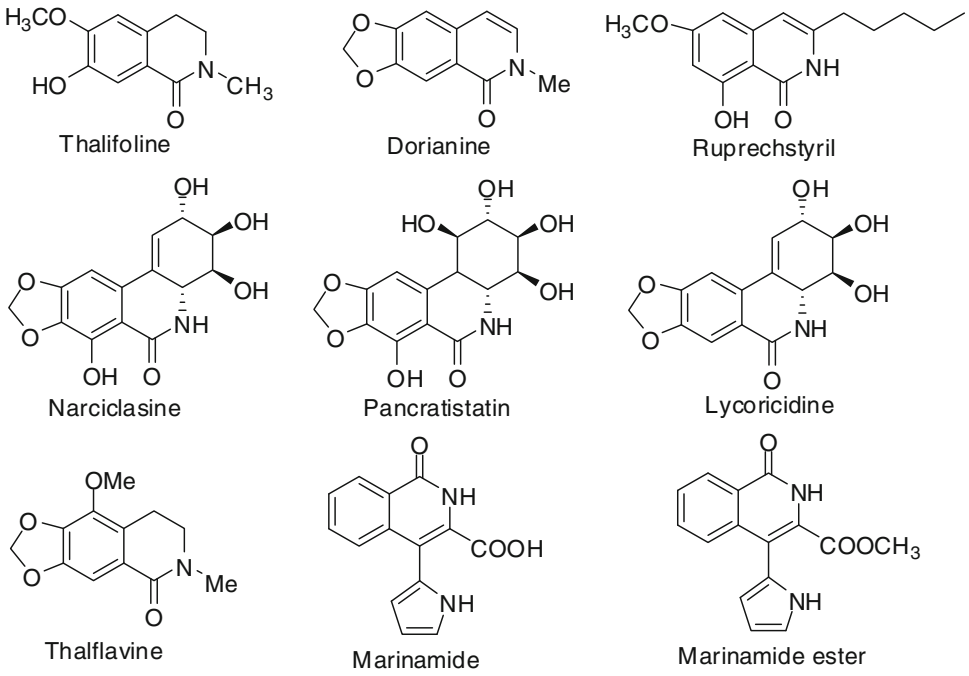


Fig. 1 Natural products containing isocarbostyril moiety

for the tissue (Paramashivappa *et al.*, 2003). However, if it is left untreated for a while, it may lead to the onset of diseases such as vasomotor rhinorrhoea, rheumatoid arthritis, and atherosclerosis (Henson and Murphy, 1989). Non-steroidal anti-inflammatory drugs (NSAIDs) are usually used to treat inflammation. These drugs act by inhibiting cyclooxygenases (COXs) which are the key enzymes involved in the biosynthesis of prostaglandin from arachidonic acid (Masferrer *et al.*, 1994; Tonk *et al.*, 2012). However, it is now believed that the presently used NSAIDs have side effects such as dyspepsia, gastrointestinal bleeding, perforation in addition to renal side effects and distinct salicylate intoxication, and are not very useful in all cases of inflammatory disorders (Polisson, 1996). Therefore, a search for safer and effective anti-inflammatory medications is necessary and needed (Katsori *et al.*, 2011; Rajakumar and Anandhan, 2011; Shafi *et al.*, 2012; Bansal *et al.*, 2013; Assarzadeh *et al.*, 2014). Herein, we report the synthesis and characterization of novel isocarbostyril derivatives as a new class of potent anti-inflammatory agents.

Experimental

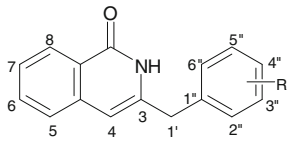
Materials and methods

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as required. Thin-layer chromatography (TLC) was performed using aluminum sheets (Merck) coated with silica gel 60 F254. Elemental analyses were carried out with a LECO-183 CHNS model. ^{1}H and ^{13}C NMR spectra of compounds were recorded with a Bruker 300 MHz spectrometer using deuterated solvents and TMS as internal standard. IR spectra of compounds were recorded on a Bio-Rad FTS 3000 MX spectrophotometer (400–4,000 cm^{-1}). The melting points of compounds were determined using capillary tubes and an electrothermal melting point apparatus, model MP-D Mitamura Riken Kogyo, Japan. *In vitro* anti-inflammatory was carried out in HEJ research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Pakistan. During the biological testing, absorbance was measured on a SpectraMax 340 microplate reader (Molecular Devices).

General procedure for the synthesis of compounds (**2a**–**2i**)

To the solution of isocoumarin (**1a**–**1i**) (0.001 mol) in 2-ethoxyethanol (10 mL) was added an excess amount (more than one equivalent) of aqueous 28 % solution of ammonia under reflux. The reaction mixture was further refluxed for another 5–8 h. The precipitates thus formed after concentration of reaction mixture on rotary evaporator were filtered by suction filtration and washed with small amount of ethanol to get crude product. The crude product was further purified by recrystallization in ethanol solvent to get TLC pure products (Fig. 2) in moderate to good yields.

Fig. 2 Labeling scheme of protons and carbons for compounds (**2a**–**2i**)



*3-(2-Fluorobenzyl)isoquinolin-1(2H)-one (**2a**)*

Yellow solid; yield, 59 %; mp = 170–172 °C; IR (KBr) υ_{max} 2760–3245, 1668, 1645 cm^{-1}; ^1H NMR (300 MHz, DMSO): $\delta = 11.42$ (s (br), 1H, NH), 8.60 (1H, d, $J = 7.8$ Hz, H-8), 7.57 (1H, dt, $J = 7.2$, 1.2 Hz, H-6), 7.45 (1H, dt, $J = 8.1$, 1.2 Hz, H-7), 7.32–7.36 (m, 2H, H-5, 6″), 7.22–7.30 (m, 3H, H-3″, 4″, 5″), 6.12 (s, 1H), 3.81 (s, 2H, C-1′); ^{13}C NMR (75 MHz, DMSO): $\delta = 162.8$ (C-1), 162.1 ($J = 245.1$ Hz, C-2″), 155.6 (C-4a), 137.4 (C-5), 134.9 (C-6), 131.9 ($J = 8.1$ Hz, C-4″), 130.8 (C-3), 129.7 (C-7), 129.1 ($J = 8.1$ Hz, C-6″), 128.6 (C-8), 125.2 (C-8a), 123.4 ($J = 3.7$ Hz, C-5″), 122.3 ($J = 15.0$ Hz, C-3″), 115.6 ($J = 21.2$ Hz, C-1″), 103.8 (C-4), 39.3 (C-1′); EIMS *m/z* 253 (24.0) $[M]^+$, 225 (12.2), 119 (33.8), 115 (26.2), 102 (6.8), 89 (100), 63 (34.2). Anal. Calcd. for $C_{16}H_{12}ONF$: C, 75.88; H, 4.78; N, 5.53. Found: C, 76.10; H, 4.94; N, 5.63.

*3-(3-Fluorobenzyl)isoquinolin-1(2H)-one (**2b**)*

Yellow solid; yield, 51 %; mp = 188–190 °C; IR (KBr) υ_{max} 2675–3190, 1670, 1643 cm^{-1}; ^1H NMR (300 MHz, DMSO): $\delta = 11.32$ (s (br), 1H, NH), 8.46 (d, 1 H, $J = 7.8$ Hz, H-8), 7.51 (dt, 1H, $J = 7.8$, $J = 1.2$ Hz, H-6), 7.43 (dt, 1H, $J = 7.2$, 0.9 Hz, H-7), 7.31–7.38 (m, 2H, H-5,5″), 7.10–7.21 (m, 3H, H-2″, 4″, 6″), 6.13 (s, 1H, H-4), 3.75 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO): $\delta = 162.8$ (C-1), 162.6 ($J = 244.5$ Hz, C-3″), 156.3 (C-4a), 138.4 ($J = 7.8$ Hz, C-1″), 137.5 (C-3), 134.4 (C-7), 130.7 ($J = 8.7$ Hz, C-5″), 129.8 (C-6), 128.4 (C-8), 125.8 (C-8a), 124.6 ($J = 3.2$ Hz, C-6″), 120.1 (C-5), 116.8 ($J = 21.0$ Hz, C-4″), 114.1 ($J = 21.0$ Hz, C-2″), 104.6 (C-4), 39.8 (C-1′); EIMS *m/z* 253 (31.0) $[M]^+$, 225 (19.0), 119 (30.8), 115 (21.9), 102 (8.8), 89 (100), 63 (31.0). Anal. Calcd. for $C_{16}H_{12}ONF$: C, 75.88; H, 4.78; N, 5.53. Found: C, 76.22; H, 4.88; N, 5.69.

*3-(4-Fluorobenzyl)isoquinolin-1(2H)-one (**2c**)*

Yellow solid; yield, 61 %; mp = 216–218C; IR (KBr) υ_{max} 2800–3200, 1667, 1644 cm^{-1}; ^1H NMR (300 MHz, DMSO): $\delta = 11.35$ (s (br), 1H, NH), 8.29 (d, 1 H, $J = 7.5$ Hz, H-8), 7.58 (dt, 1H, $J = 7.5$, 1.2 Hz, H-6), 7.42 (dt, 1H, $J = 8.1$, 1.2 Hz, H-7), 7.22–7.30 (m, 3H, H-5, 2″, 6″), 7.01–7.02 (m, 2H, H-3″, 5″), 6.0 (s, 1H, H-4), 3.80 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO): $\delta = 162.9$ (C-1), 162.3 ($J = 245.5$ Hz, C-4″), 156.8 (C-3), 137.1 (C-4a), 134.4 (C-6), 131.1 ($J = 3.2$ Hz, C-1″), 130.2 ($J = 8.5$ Hz, C-2″, 6″), 129.8 (C-8a), 127.2 (C-7), 125.8 (C-8), 120.2 (C-5), 115.3 ($J = 21.5$ Hz, C-3″, 5″), 103.6 (C-4), 39.2 (C-1′); EIMS: *m/z* 253 (21.0) $[M]^+$, 225 (14.9), 119 (22.1), 115 (29.0), 102 (7.2), 89 (100), 63 (29.0); Anal. Calcd. for $C_{16}H_{12}ONF$: C, 75.88; H, 4.78; N, 5.53. Found: C, 76.28; H, 4.84; N, 5.68.

*3-(2-Chlorobenzyl)isoquinolin-1(2H)-one (**2d**)*

Yellow solid; yield, 52 %; mp = 219–221 °C; IR (KBr) υ_{max} 2730–3148, 1674, 1643, 1049 cm^{-1}; ^1H-NMR (300 MHz, DMSO): $\delta = 11.31$ (1H, s, NH), 8.14 (1H, d, $J = 8.1$ Hz), 7.48–7.51 (2H, m), 7.40–7.45 (2H, m), 7.31–7.37 (3H, dt, $J = 7.5$, 1.2, Hz), 6.01 (1H, s), 3.99 (2H, s); ^{13}C NMR (75 MHz, DMSO): $\delta = 163.4$ (C-1), 142.6 (C-1″), 139.9 (C-4a), 137.5 (C-6), 132.5 (C-3″), 131.4 (C-6″), 131.1 (C-3), 130.6 (C-7), 128.7 (C-4″), 128.3 (C-5″), 128.0 (C-8), 127.4 (C-8a), 126.3 (C-5), 124.9 (C-2″), 104.9 (C-4), 38.5 (C-1′); EIMS: *m/z* 269 (43.0) $[M]^+$, 234 (100), 233 (15.2), 204 (8.7), 178 (5.9), 125 (9.3), 115 (11.0), 90 (13.1), 89 (87.5), 63 (25.2); Anal. Calcd. for $C_{16}H_{12}ONCl$: C, 71.37; H, 4.46; N, 5.20. Found: C, 71.26; H, 4.55; N, 5.06.

*3-(3-Chlorobenzyl)isoquinolin-1(2H)-one (**2e**)*

Yellow solid; yield, 45 %; mp = 200–201 °C; IR (KBr) υ_{max} 2810–3216, 1663, 1641, 1083 cm^{-1}; ^1H NMR (300 MHz, DMSO): $\delta = 11.41$ (1H, s, NH), 8.12 (1H, d, $J = 8.4$ Hz, H-8), 7.65 (1H, dt, $J = 7.5$, 1.5 Hz, H-6), 7.55 (1H, d, $J = 7.5$ Hz, H-7), 7.46 (1H, d, $J = 7.5$ Hz, H-5), 7.42 (1H, dt, $J = 8.1$, 1.5 Hz, H-5″), 7.29–7.36 (3H, m, H-2″, 4″, 6″), 6.38 (1H, s, H-4), 3.84 (2H, s, H-1′); ^{13}C NMR (75 MHz, DMSO): $\delta = 162.9$, 141.4, 141.0, 138.4, 133.5, 132.9, 130.8, 129.2, 128.1, 127.1, 127.0, 126.4, 126.3, 124.9, 103.8, 38.1; EIMS: *m/z* 269 (96.4) $[M]^+$, 234 (14.2), 233(12.8), 204 (9.5), 178 (4.9), 125 (8.6), 115 (14.2), 90 (22.2), 89(100), 63 (35.0); Anal. Calcd. for $C_{16}H_{12}ONCl$: C, 71.37; H, 4.46; N, 5.20. Found: C, 71.49; H, 4.61; N, 5.33.

*3-(4-Chlorobenzyl)isoquinolin-1(2H)-one (**2f**)*

Yellow solid; yield, 43 %; mp = 231–233 °C; IR (KBr) υ_{max} 2680–3210, 1672, 1644, 1093 cm^{-1}; ^1H NMR (300 MHz, DMSO): $\delta = 11.40$ (1H, s, NH), 8.12 (1H, d, $J = 7.8$ Hz, H-8), 7.64 (1H, t, $J = 7.5$, Hz, H-6), 7.54 (1H, d, $J = 7.8$ Hz, H-7), 7.38–7.44 (5H, m, H-5,2″, 4″, 5″, 6″),

6.32 (1H, s, H-4), 3.83 (2H, s, H-1′); ^{13}C NMR (75 MHz, DMSO): δ = 162.9, 141.7, 138.5, 137.5, 132.9, 131.8, 131.2, 128.9, 127.0, 126.4, 126.3, 124.9, 103.7, 37.8; EIMS: *m/z* 269 (69.0) $[M]^+$, 234 (12.2), 233 (11.8), 204 (6.2), 178 (6.0), 125 (18.5), 115 (9.2), 90 (21.9), 89 (100), 63 (24.2); Anal. Calcd. for $C_{16}H_{12}ONCl$: C, 71.37; H, 4.46; N, 5.20. Found: C, 71.42; H, 4.37; N, 5.31.

*3-(2-Bromobenzyl)isoquinolin-1(2H)-one (**2g**)*

Yellow solid; yield, 54 %; mp = 180–182 °C; IR (KBr) υ_{max} 2750–3250, 1669, 1640 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 11.46 (s (br), 1H, NH), 8.14 (d, J = 8.1 Hz, 1H, H-8), 7.67 (d, J = 7.8 Hz, 1H, H-6), 7.62 (dt, J = 7.2,1.2 Hz, 1H, H-7), 7.39–7.50 (m, 2H, H-5, 6″), 7.23–7.29 (m, 3H, H-2″, 4″, 5″), 5.97 (s, 1H, H-4), 3.98 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO): δ = 162.9 (C-1), 140.5 (C-1″), 138.3 (C-4a), 137.1 (C-6), 133.2 (C-3″), 132.9 (C-6″), 131.8 (C-3), 129.5 (C-7), 128.5 (C-4″), 127.0 (C-5″), 126.3 (C-8), 126.3 (C-8a), 124.9 (C-5), 124.8 (C-2″), 103.5 (C-4), 38.7 (C-′); EIMS: *m/z* 315 (22.5) $[M + 2]^+$, 313 (22.5) $[M]^+$, 234 (78.3), 233 (14.1), 217 (2.7), 216 (5.6), 206 (4.3), 205 (2.8), 204 (8.2), 119 (33.7), 115 (17.3), 102 (6.7), 89 (100), 63 (34.46). Anal. Calcd. for $C_{16}H_{12}ONBr$: C, 61.17; H, 3.85; N, 4.46. Found: C, 61.54; H, 3.74; N, 4.86.

*3-(3-Bromobenzyl)isoquinolin-1(2H)-one (**2h**)*

Yellow solid; yield, 57 %; mp = 197–200 °C; IR (KBr) υ_{max} 2680–3200, 1675, 1647 cm^{-1}. ^{1}H NMR (300 MHz, DMSO): δ = 11.41 (s (br), 1H, NH), 8.12 (d, J = 7.8 Hz, 1H, H-8), 7.65 (t, J = 7.8 Hz, 1H, H-6), 7.54–7.60 (m, 2H, H-7,5), 7.36–7.46 (m, 3H, H-2″, 4″, 6″), 7.29 (t, J = 7.8, 1H, H-5″), 6.38, (s, 1H, H-4), 3.84 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO): δ = 162.9 (C-1), 141.4 (C-1″), 141.3 (C-4a), 138.4 (C-2″), 132.9 (C-5″), 132.0 (C-4″), 131.1 (C-6), 130.0 (C-3), 128.4 (C-7), 127.0 (C-6″), 126.4 (C-8a), 126.3 (C-8), 124.9 (C-5), 122.1 (C-3″), 103.7 (C-4), 38.1 (C-1′); EIMS: *m/z* 315 (42.5) $[M + 2]^+$, 313 (42.8) $[M]^+$, 234 (17.9), 233 (18.3), 217 (13.8), 216 (5.6), 206 (3.1), 205 (2.6), 204 (9.5), 119 (16.6), 115 (11.5), 102 (10.9), 89 (100), 63 (23.8). Anal. Calcd. for $C_{16}H_{12}ONBr$: C, 61.17; H, 3.85; N, 4.46. Found: C, 61.15; H, 3.74; N, 4.71.

*3-(4-Bromobenzyl)isoquinolin-1(2H)-one (**2i**)*

Yellow solid; yield 51 %; m.p = 236–238 °C; IR (KBr) υ_{max} 2800–3200, 1667, 1644 cm^{-1}; ^{1}H NMR (300 MHz, DMSO): δ = 11.39 (s (br), 1H, NH), 8.12 (d, J = 7.8 Hz, 1H, H-8), 7.65 (dt, J = 7.2, 1.5 Hz, 1H, H-6), 7.54 (d, J = 8.4 Hz, 2H, H-2″, 6″), 7.46 (d, J = 8.1, 1H, H-7), 7.41 (dt, J = 8.1,1.2, 1H, H-5), 7.31 (d, J = 8.4 Hz, 2H, H-3″, 5″), 6.32 (s, 1H, H-4), 3.82 (s, 2H, H-1′); ^{13}C NMR (75 MHz, DMSO): δ = 162.9 (C-1), 141.6 (C-1″), 138.4 (C-4a), 137.9 (C-6), 132.9 (C-3), 131.8 (C-2″, 6″), 131.6 (C-3″, 5″), 126.3 (C-7), 126.3 (C-8a), 124.8 (C-8), 120.8 (C-4″) 120.2 (C-5), 103.7 (C-4), 37.9 (C-1′); EIMS: *m/z* 315 (27.6) $[M + 2]^+$, 313 (27.6) $[M]^+$, 234 (14.2), 233 (18.8), 217 (10.17), 216 (6.9), 206 (3.0), 205 (2.5), 204 (8.6), 119 (12.4), 115 (12.7), 102 (9.7), 89 (100), 63 (32.7); Anal. Calcd. for $C_{16}H_{12}ONBr$: C, 61.17; H, 3.85; N, 4.46. Found: C, 61.48; H, 3.66; N, 4.87.

Anti-inflammatory bioassay (in vitro)

Isolation of human neutrophils

Heparinized fresh venous blood was drawn from healthy volunteers in a local blood bank and neutrophils were isolated (Siddiqui *et al.*, 1995).

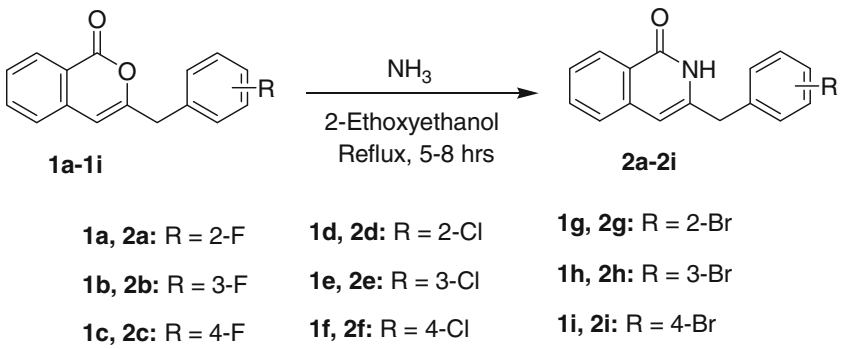
Respiratory burst assay

Anti-inflammatory activity of the synthesized compounds was determined using a modified assay (Tan and Berridge, 2000). This in vitro assay was based on the reduction of highly water-soluble tetrazolium salt (WST-1) in the presence of activated neutrophils. Anti-inflammatory activity was determined in a total volume of 200-µL MHS (pH 7.4) containing 1.0–104 neutrophils/mL, 250-µM WST-1, and various concentrations of test-synthesized compounds. The control contained buffer, neutrophils, and WST-1. All the synthesized compounds were equilibrated at 37 °C, and the reaction was initiated by adding opsonized zymosan A (15 mg/mL), which was prepared by mixing with human pooled serum, followed by centrifugation at 3,000 rpm, and the pellet was resuspended in PBS buffer. Absorbance was measured at 450 nm. Aspirin and indomethacin were used as positive controls which are widely used as non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of several inflammatory diseases. Values of IC_{50} were calculated by comparison with the DMSO as the blank and expressed as the percent inhibition of superoxide anions produced. The percent inhibitory activity by the samples was determined against a DMSO blank and calculated using the following formula:

$$\%\,\text{Inhibition} = 100 - \{(\text{OD test compound}/\text{OD control}) \times 100\}$$

IC_{50} of samples was determined using EZ-FIT Windows-based software.

Scheme 1 Synthesis of new isocarbostyrils/3-(halobenzyl)isoquinolinones (**2a–2i**)



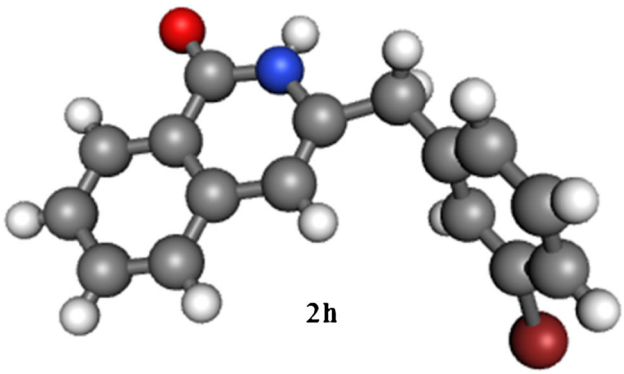


Fig. 3 Crystalline structure of 3-(3-Bromobenzyl)isoquinolin-1(2*H*)-one (**2h**)

Results and discussion

Chemistry

Nine new isocarbostyrils/isoquinolinones (**2a–2i**) were synthesized by treating the solution of isocoumarins (**1a–1i**) (Abid *et al.*, 2010) in 2-ethoxyethanol with 28 % aqueous ammonia for 5–8 h under reflux conditions (Scheme 1) and purified by washing of the obtained precipitates with small amount of ethanol and later by recrystallization in ethanol solvent. The structures of TLC pure compounds (**2a–2i**) isolated in 43–61 % yields were established on the basis of their spectroscopic (IR, ^{1}H NMR, and ^{13}C NMR, Mass) and microanalysis data. In addition, the crystalline structure of one of the compounds of the present series **2a–2i** has recently been reported (Ali *et al.*, 2010) (Fig. 3).

Spectroscopic characterization of compounds

IR spectra

In the IR spectra of compounds (**2a–2i**), the carbonyl (C=O) stretching absorption was observed at 1,663–1,675 cm^{-1}, a value lower than that of isocoumarins (**1a–1i**) (Abid *et al.*, 2010) indicating the formation of amide from cyclic ester. In addition, a broad band in the region of 2,675–3,250 cm^{-1} and a sharp band at 1,640–1,647 cm^{-1} due to N–H stretching and bending absorptions, respectively, further confirmed the formation of products (**2a–2i**).

NMR spectra

The resonance of protons has been assigned on the basis of their integration and multiplicity pattern. The most characteristic signal was observed in the region of 11.32–11.46 ppm assigned to NH protons. Another important peak was observed at 5.97–6.38 ppm allocated to H-4 protons appeared in the upfield region as compared to isocoumarins (**2a–2i**). Both these peaks were indicative of the formation of the products (**2a–2i**) from **1a–1i**. All other peaks were found in their expected regions. Moreover, the number of the protons calculated from the integration curves and those obtained from the values of the CHN analysis were in good agreement also suggesting the formation of the target compounds. The ^{13}C NMR spectra provided further support to the information obtained from the IR and ^{1}H NMR spectral data. The carbonyl carbon for the compounds (**2a–2i**) was appeared in the region of 162.2–162.9 ppm. All other carbon signals were observed in their respective regions. The complete details of all the signals observed in ^{1}H and ^{13}C NMR spectra can be seen in the experimental section.

Mass spectra

The electron impact mass spectra (EIMS) of the all the synthesized isocarbostyrils (**2a–2i**) are presented in the experimental part. The mass spectral data and fragmentation pattern of all the synthesized compounds clearly justify the formation of proposed structures discussed in IR, ^{1}H and ^{13}C NMR spectroscopy. Further, a molecular ion peak ($M^{+\bullet}$) was observed for all the compounds at their respective molecular masses. The most stable fragment or base peak was observed at *m/z* 89 for 1H-cyclopropabenzen-1-ylium cation for all the compounds.

Pharmacology

Anti-inflammatory studies (in vitro)

It is an established fact that inflammation occurs as a defensive response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, which induces physiological adaptations to minimize damage. This defensive process is initiated by the activation of phagocyte-specific enzyme, NADPH oxidase which generates superoxide anion (a reactive free radical) by transferring electrons from NADPH to molecular oxygen inside the cell across the membrane. Superoxide kills bacteria and fungi by still unknown mechanisms, a key step of immune response and inflammatory cascade. Further, the superoxide can spontaneously form hydrogen peroxide that may undergo further reactions to produce reactive oxygen species (ROS). Inhibition of highly toxic ROS formed in large quantities in pathological conditions is an approach to treat chronic inflammation and to modulate immune response in immune-compromised patients. In the present study, we used the water-soluble tetrazolium salt (WST-1) to measure the superoxide produced by neutrophils (Tan and Berridge, 2000). This technique is more sensitive and reliable to measure the superoxide scavenging properties as compared to other available techniques, a perfect protocol for indirect evaluation of anti-inflammatory potential.

Anti-inflammatory potential of the synthesized isocarbostyrils **2a**–**2i** was determined by measuring their effect on superoxide production (Tan and Berridge, 2000). The anti-inflammatory activity was first evaluated in terms of percent growth inhibition, and the compounds with ≥50 % inhibition were retested for their IC50 (inhibitory concentration 50 %), the concentration of the compound which inhibits superoxide production by 50 % of three independent experiments (Table 1). Indomethacin (a commercially available NSAID) was used as the standard reference (SR) drug in this study. Interestingly, only three compounds **2c**, **2f**, and **2i** bearing halide substituents on *para*-position of benzyl group of isocarbostyril skeleton, showed more than 50 % inhibition and were considered to be active. All other compounds **2a**, **2b**, **2d**, **2e**, **2g**, and **2h** having halide substituents on *ortho- and meta*-position of benzyl group of isocarbostyril skeleton showed less than 50 % inhibition were not screened for their IC50 values and considered to be as inactive. Among the three active compounds, **2c** (IC50 = 251.002 ± 2.910) showed the best anti-inflammatory activity, which is comparable to Indomethacin (IC50 = 271.210 ± 2.127). Other two compounds, **2f** (IC50 = 329.809 ± 4.125) and **2i** (IC50 = 391.217 ± 6.251) also showed good anti-inflammatory potential.

From these results, it can be concluded that the low activity of 3-(*o*-halobenzyl)isocarbostyrils **2a**, **2d**, and **2g** may be due to the maximum possible secondary electronic interactions between halides and N–H of amide functionality resulting in the closure of active pharmacophore site. The absence of this factor and maximum possibility of secondary electronic interactions with the positively charged side chains of the target(s) due to the presence of highly electronegative fluoro-substituent at *para*-position of benzyl group led to excellent anti-inflammatory activity for compound **2c**.

Table 1 Anti-inflammatory activity of isocarbostyrils (**2a**–**2i**)

Compound	X	% inhibition	IC_{50} ± SEM (μM)
2a	2-F	35.3	–
2b	3-F	40.4	–
2c	4-F	89.2	251.002 ± 2.910
2d	2-Cl	21.1	–
2e	3-Cl	32.7	–
2f	4-Cl	85.2	329.809 ± 4.125
2g	2-Br	20.3	–
2h	3-Br	39.3	–
2i	4-Br	81.4	391.217 ± 6.251
Indomethacin	–	91.0	**271.210 ± 2.127**

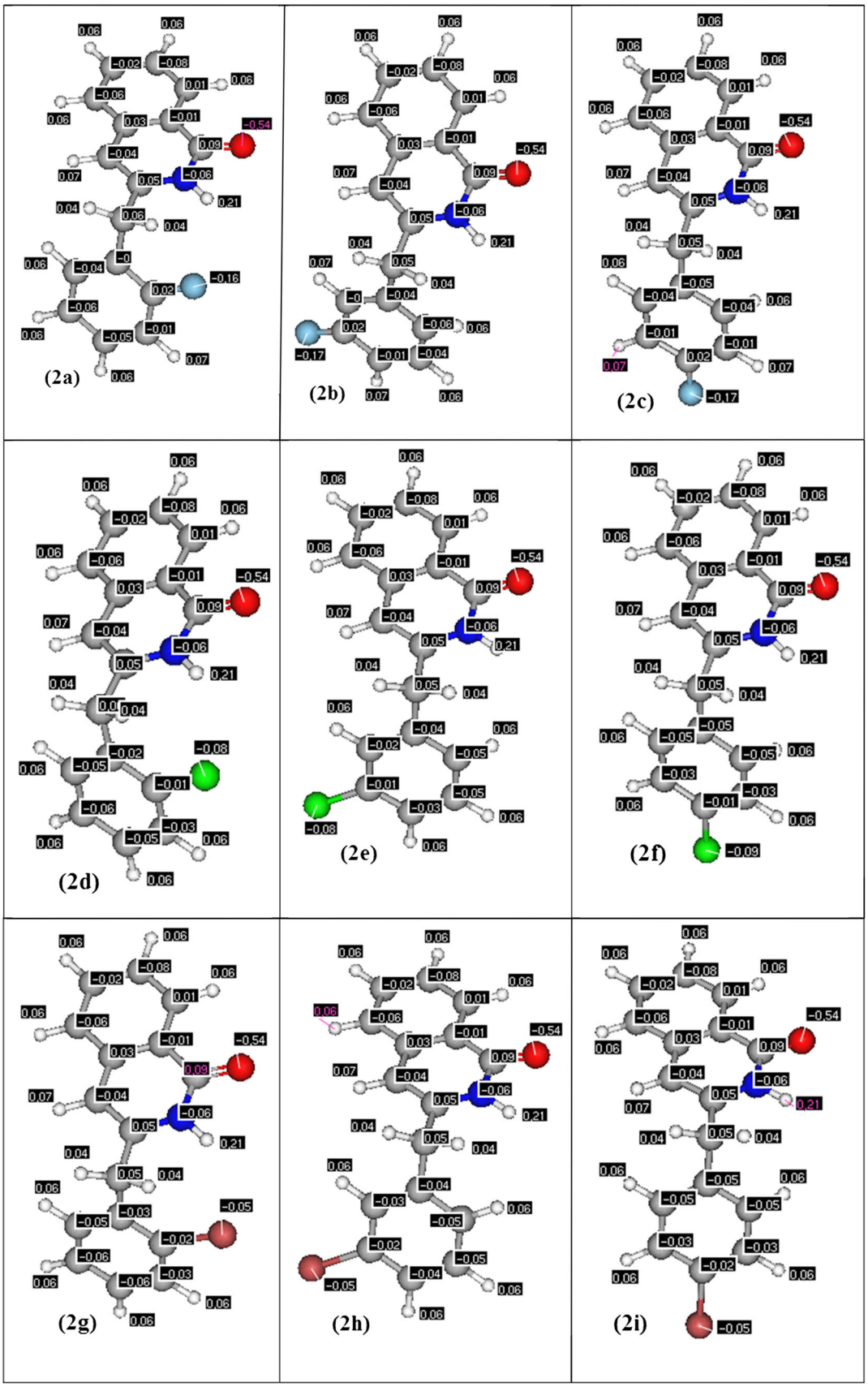
A computational Petra/Osiris/Molinspiration (POM) analytical approach was then used to explore structural features that are necessary for anti-inflammatory activity.

Molecular properties calculations

To understand anti-inflammatory action of compounds **2a**–**2i**, the identification of the active structural features is important. Calculations of energetics, atomic charges, minimum energy structures, geometry, and natural bond orbital (NBO) could indicate the electronic density distribution of each atom. These systematic data, regarding the variation of molecular properties with a small change in chemical structure, therefore, provide first insights into the chemical bonding of isocarbostyrils with various targets. In brief, the objective of this study is to identify and investigate the potential pharmacophore sites of isocarbostyrils **2a**–**2i** responsible for their bioactivity through molecular properties calculations by POM analyses.

Petra calculations

PETRA is a program package comprising of various empirical methods for the calculation of physicochemical properties of organic molecules. All methods are empirical in nature and have been developed over the last 20 years in the research group of Gasteiger *et al.* (1985). The following chemical effects can be quantified: heats of formation, bond dissociation energies, sigma charge distribution, total/


(2a)
(2b)
(2c)
(2d)
(2e)
(2f)
(2g)
(2h)
(2i)

◀**Fig. 4** Total charge distribution of series **2a–2i**. All compounds with halide substituent on C-2 position of aryl are in cisoidal conformation which clearly indicates the reason of decrease in anti-inflammatory activity of **2a**, **2d** and **2g** in comparison to rest of the compounds of the series **2a–2i**

Table 2 Total charges' calculations of compounds **2a–2i**

Compd.	X	N and O hetero atoms				Protons of terminal aryl		
		O-1	N	X	N–H	H–C-2	H–C-3	H–C-4
2a	2-F	−5.54	−0.006	−0.16	0.21	–	0.07	0.06
2b	3-F	−0.54	−0.06	−0.17	0.21	0.07	–	0.07
2c	4-F	−0.54	−0.06	−0.17	0.21	0.06	0.07	–
2d	2-Cl	−0.54	−0.06	−0.08	0.21	–	0.06	0.06
2e	3-Cl	−0.54	−0.06	−0.08	0.21	0.06	–	0.06
2f	4-Cl	−0.54	−0.06	−0.09	0.21	0.06	0.06	–
2g	2-Br	−0.54	−0.06	−0.05	0.21	–	0.06	0.06
2h	3-Br	−0.54	−0.06	−0.05	0.21	0.06	–	0.06
2i	4-Br	−0.54	−0.06	−0.05	0.21	0.06	0.06	–

π-charge distribution, inductive effect, resonance effect and delocalization energies, and polarizability effect.

The present series of isocarbostyrils **2a–2i** have been subjected to total and delocalised-charge calculations using Petra method of the non-hydrogen common atoms (Fig. 4), obtained from the total charge of the hetero atoms to model the bioactivity (Table 2). It is found that in case of compounds **2a**, **2d**, and **2g** having halide substituents on 2-position of terminal aryl, the decrease in bioactivity is due to the presence of intramolecular hydrogen bonding interactions between amide N–H and halide substituents. These interactions play negative role in the bioactivity by closing the amide pharmacophore site (Rajakumar *et al.*, 2006) as shown in Fig. 5. In contrast, according to our total and delocalised-charge calculations, other compounds of the series bearing halide substituents at 3- and 4-positions have no such interactions and can be expected to have better activity, which is in very good agreement with our experimental results. The excellent anti-inflammatory activity of compound **2c** may be attributed to the presence of highly electronegative fluoro-substituent at C-4 position due to which it has maximum room for secondary electronic interactions with the positively charged side chains of the target(s). In addition, the results of the present study support the previous findings (Waring *et al.*, 2002) where aryl or heterocyclic ring with potential hydrogen bond acceptor substituents present near to N–H (amine) moiety could generate some unwanted interactions which led to the decrease in bioactivity (Fig. 6).

Osiris calculations

Structure-based design is now fairly routine, but many potential drugs fail to reach the clinical trials because of ADME-Tox liabilities. One very important class of enzymes, responsible for many ADMET problems, is the cytochromes P450. Many drugs may increase or decrease the activity of this enzyme either by inducing the biosynthesis of an isozyme or by directly inhibiting its activity resulting in many adverse drug reactions with the production of unwanted metabolites. One of the most important online available program, Osiris which uses a combined

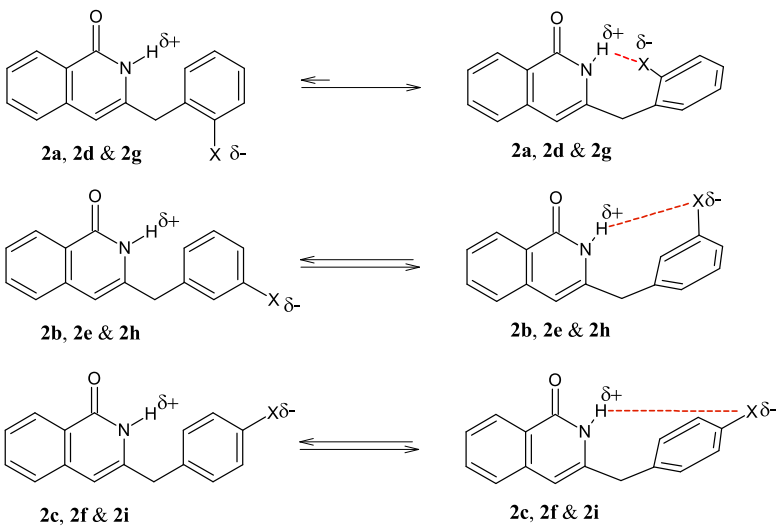


Fig. 5 Impact of position of halide substituent (X) on pharmacophore site. The intramolecular N–H–X is stronger in compounds (**2a**, **2d**, **2g**) than their analogs with X at C-3 or C-4 positions

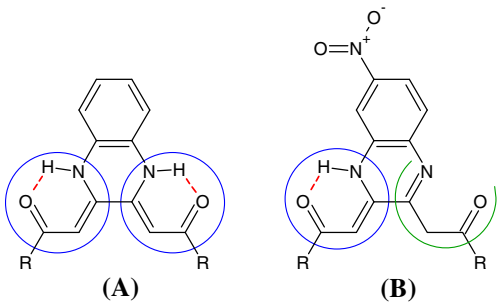


Fig. 6 Impact of intramolecular hydrogen bond interactions on bioactivity; the compounds of series (**b**) are relatively more active than series (**a**) against microorganisms (Rajakumar *et al.*, 2006)

electronic/structure docking procedure is very helpful in the structure-based design of drugs to avoid such adverse reactions at a later stage. The remarkable well-behaved mutagenicity of diverse synthetic molecules classified in data base of CELERON Company of Swiss can be used to quantify the role played by various organic groups in promoting or interfering with the way a drug can associate with DNA.

The data in Table 3 indicate that all the structures **2a**–**2i** are supposed to be non-mutagenic when run through the mutagenicity assessment system and as far as irritating and reproductive effects are concerned, all the compounds (except **2b**) are at low risk comparable with the standard drug used. The Clog P value of a compound, which is the logarithm of its partition coefficient between *n*-octanol and water, is a well-established measure of the compound's hydrophilicity. Low hydrophilicity, and therefore, high-Clog P values may cause poor absorption or permeation. It has been proposed that Clog P value for compounds must not be greater than 5.0 to have a reasonable probability of being well absorbed. The Clog P values of compounds **2a–2i** are well under the acceptable criteria. The geometrical parameters and the aqueous solubility of a compound significantly affect its absorption, distribution characteristics, and bioactivity. Typically, a low solubility goes along with a bad absorption, and therefore, the general aim is to avoid poorly soluble compounds.

Overall drug-score (DS) for the compounds **2a**–**2i** was calculated and compared with that of standard drug Indomethacin. The DS based on drug-likeness (DL), CLogP, logS, molecular weight, and toxicity risks is one handy value that may be used to judge the compound's overall potential to qualify for a drug. All the compounds of the present series **2a–2i** have good DS which indicates that majority of parameters in drug design have been realized.

Molinspiration calculations

Modern drug discovery is based in large part on high-throughput screening of small molecules against macromolecular disease targets which require druglike or lead-like compounds in molecular screening libraries. After analyzing known SR for drug-like and lead-like properties, the specific drug-like or lead-like compounds **2a**–**2i** were designed. DL of these compounds is calculated by their virtual screening against various enzymes using Molinspiration methodology (Table 4).

The polar surface area (PSA) is calculated from the surface areas that are occupied by oxygen, nitrogen, and any other hetero atoms and by hydrogen atoms attached to them by methodology published by Ertl *et al.* (2000). Thus, the PSA expresses the hydrogen bonding potential of a compound to some extent (Clark, 1999). TPSA has been

Table 3 Osiris calculations of toxicity risks and DS of compounds **2a**–**2i**

Compd.	MW	Toxicity risks				Drug-score			
		MUT	TUMO	IRRI	REP	CLP	S	DL	DS
2a	253	nt	nt	nt	nt	3.36	−4.26	0.13	0.58
2b	253	nt	nt	nt	st	3.36	−4.26	−1.57	0.35
2c	253	nt	nt	nt	nt	3.36	−4.26	0.46	0.61
2d	269	nt	nt	nt	nt	3.92	−4.68	1.93	0.63
2e	269	nt	nt	nt	nt	3.92	−4.68	1.07	0.58
2f	269	nt	nt	nt	nt	3.92	−4.68	2.43	0.64
2g	313	nt	nt	nt	nt	4.00	−4.78	−2.19	0.35
2h	313	nt	nt	nt	nt	4.00	−4.78	−2.14	0.35
2i	313	nt	nt	nt	nt	4.00	−4.78	−0.61	0.45
SD	357	nt	nt	nt	nt	3.83	−5.4	7.59	0.57

nt not toxic, *st* slightly toxic, *MUT* mutagenic, *TUMO* tumorigenic, *IRRI* irritant, *REP* reproductive effective, *CLP* Clog P, *S* solubility, *DL* drug-likeness, *DS* drug-score, *SD* indomethacin

Table 4 Molinspiration calculations and drug-score of compounds **2a–2i**

Compd.	Physico-chemical properties					Drug-likeness					
	TPSA	O/NH	VIOL	ROTB	VOL	GPC	ICM	KI	NRL	PI	EI
2a	33	1	0	2	225	−0.06	0.01	0.08	−0.29	−0.27	0.24
2b	33	1	0	2	225	−0.01	0.04	0.11	−0.23	−0.14	0.26
2c	33	1	0	2	225	−0.02	0.03	0.11	−0.24	−0.17	0.25
2d	33	1	0	2	234	−0.05	−0.01	0.03	−0.33	−0.24	0.15
2e	33	1	0	2	234	−0.02	0.05	0.03	−0.31	−0.20	0.23
2f	33	1	0	2	234	−0.03	0.04	0.04	−0.30	−0.20	0.23
2g	33	1	0	2	238	−0.14	0.02	0.04	−0.37	−0.30	0.18
2h	33	1	0	2	238	−0.17	−0.06	−0.03	−0.45	−0.32	0.16
2i	33	1	0	2	238	−0.16	−0.05	0.01	−0.42	−0.29	−0.18
SD	67	1	0	4	303	0.24	−0.31	−0.11	0.42	−0.11	0.30

TPSA total molecular polar surface area, *OHN* number of N–H–O interaction, *VIOL.* number of violation of five Lipinsky rules, *VOL.* volume, *GPC* GPCR ligand, *ICM* ion channel modulator, *KI* kinase inhibitor, *NRL* nuclear receptor ligand, *PI* protease inhibitor, *EI* enzyme inhibitor, *SD* indomethacin

shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability, and blood–brain barrier penetration. Molecules with TPSA values around of 160 Å or more are expected to exhibit poor-intestinal absorption (Clark, 1999). Table 4 shows that all the compounds are under this limit. It has to be kept in mind that log *P* and PSA values are only two important, although not sufficient criteria for predicting oral absorption of a drug (Viswanadhan *et al.*, 1989).

Oral bioavailability is a desirable property of compounds under investigation in the drug discovery process. Lipinski′s rule-of-five is a simple model to forecast the absorption and intestinal permeability of a compound (Lipinski *et al.*, 2001). In the rule-of-five model, compounds are considered likely to be well absorbed when they possess these attributes—molecular weight <500, cLogP <5, number of H-bond donors <5, number H-bond acceptors <10, and number of rotatable bonds <10.

DL of compounds (**2a**)–(**2i**) is tabulated in Table 4. DL may be defined as a complex balance of various molecular properties and structure features which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility, and presence of various pharmacophore features influence the behavior of molecule in a living organism, including bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability, and many others. Activity of all nine compounds (**2a**–**2i**) and standard drug were rigorously analyzed under four criteria of known successful drug activity in the areas of GPCR ligand activity, ion channel modulation, kinase inhibition activity, and nuclear receptor ligand activity, and the results are shown for all compounds in Table 4 by means of numerical assignment.

Conclusions

In conclusion, we have synthesized a series of novel 3-(halobenzyl)isocarbostyrils and characterized them on the basis of spectral data and microanalysis. All the synthesized compounds were then screened for anti-inflammatory properties and their structure–activity relationship was established. It has been found that the position of halide substituents displayed a significant role on bioactivity in addition to their high electronegativity (increases polarity, hydrophilic character, and biological absorbance). The isocarbostyril derivatives bearing halide substituents on *ortho*-position of benzyl group showed the lowest activity perhaps due to the closure of pharmacophore site by intramolecular hydrogen bond between halides and N–H of amide. In contrast, isocarbostyrils **2c**, **2f,** and **2i** having halides substituents at *para*-position where there was no such interaction exhibited moderate to very good inflammatory activity. Compound **2c** (IC_{50} = 251.002 ± 2.910) was found to be the most potent compound of the series having activity comparable to standard drug (Indomethacin, IC_{50} = 271.210 ± 2.127). Theoretical computational POM studies also confirm our proposition for low activity of *ortho*-substituted 3-(halobenzyl)isocarbostyrils. Furthermore, POM analyses insinuate constructive hints for the design of new analogs with enhanced biological activities, and therefore on the basis of their structural properties, these compounds may serve as powerful lead compounds for future studies.

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References

Abid OUR, Babar TM, Ali FI, Ahmed S, Wadood A, Rama NH, Uddin R, Zaheer-ul-Haq, Khan A, Choudhary MI (2010) Identification of novel urease inhibitors by high-throughput virtual and in vitro screening. ACS Med Chem Lett 1:145–149

Ali FI, Babar TM, Rama NH, Jones PG (2010) 3-(3-Bromobenzyl)isoquinolin-1(2*H*)-one. Acta Crystallogr E 66:O16–O1225

Aly Y, Galal A, Wong LK, Fu EW, Lin FT, Duah FK, Schiff PL (1989) A revision of the structure of the isoquinolone alkaloid thalflavine. Phytochem 28:1967–1971

Asano Y, Kitamura S, Ohra T, Itoh F, Kajino M, Tamura T, Kaneko M, Ikeda S, Igata H, Kawamoto T, Sogabeb S, Matsumotob S, Tanakab T, Yamaguchib M, Kimurab H, Fukumoto S (2008) Discovery, synthesis and biological evaluation of isoquinolones as novel and highly selective JNK inhibitors (2). Bioorgan Med Chem 16:4699–4714

Assarzadeh MJ, Almasirad A, Shafiee A, Koopaei MN, Abdollahi M (2014) Synthesis of new thiazolo[3,2-*b*][1,2,4]triazole-6(5*H*)-one derivatives as potent analgesic and anti-inflammatory agents. Med Chem Res 23:948–957

Bansal Y, Sethi P, Bansal G (2013) Coumarin: a potential nucleus for anti-inflammatory molecules. Med Chem Res 22:3049–3060

Clark DE (1999) Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 1. Prediction of intestinal absorption. J Pharm Sci 88:807–814

Coelho F, Veronese D, Lopes ECS, Rossi RC (2003) An approach to substituted dihydroisoquinolin-1(2*H*)-ones from Baylis–Hillman adducts. Tetrahedron Lett 44:5731–5735

Ertl P, Rohde B, Selzer P (2000) Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 43:3714–3717

Gasteiger J, Hutchings MG, Marsili M, Saller H (1985) Rapid calculation of electronic effects in organic molecules. In: Seydel JK (ed) QSAR and strategies in the design of bioactive compounds. VCH-Verlagsgesellschaft, Weinheim, pp 90–97

Glushkov VA, Shklyaev YV (2001) Synthesis of 1(2*H*)-isoquinolones (review). Chem Heterocycl Compd 37:663–687

Guastavino JF, Barolo SM, Rossi RA (2006) One-pot synthesis of 3-substituted isoquinolin-1-(2*H*)-ones and fused isoquinolin-1-(2*H*)-ones by SRN1 reactions in DMSO. Eur J Org Chem: 3898–3902

Heaney H, Shuhaibar KF (1995) New routes to acyliminium ion precursors and a synthesis of the nuevamine skeleton. Synlett 1995:47–48

Henson PM, Murphy RC (1989) Mediators of the inflammatory process, 6th edn. Elsevier, Amsterdam

Hudlicky T, Rinner U, Gonzalez D, Akgun H, Schilling S, Siengalewicz P, Martinot TA, Pettit GR (2002) Total synthesis and biological evaluation of amaryllidaceae alkaloids: narciclasine, ent-7-deoxypancratistatin, regioisomer of 7-deoxypancratistatin, 10b-epi-deoxypancratistatin, and truncated derivatives. J Org Chem 67:8726–8743

Katsori AM, Chatzopoulou M, Dimas K, Kontogiorgis C, Patsilinakos A, Trangas T, Hadjipavlou-Litina D (2011) Curcumin analogues as possible anti-proliferative and anti-inflammatory agents. Eur J Med Chem 46:2722–2735

Kohlhagen G, Paull KD, Cushman M, Nagafuji P, Pommier Y (1998) Protein-linked DNA strand breaks induced by NSC 314622, a novel noncamptothecin topoisomerase I poison. Mol Pharmacol 54:50–58

Krane BD, Shamma M (1982) The isoquinolone alkaloids. J Nat Prod 45:377–384

Li LH, Chua WKS (2011) One-pot multistep synthesis of 3,4-fused isoquinolin-1(2*H*)-one analogs. Tetrahedron Lett 52:1574–1577

Li SW, Nair MG, Edwards DM, Kisliuk RL, Gaumont Y, Dev IK, Duch DS, Humphreys J, Smith GK, Ferone R (1991) Folate analogs.35. synthesis and biological evaluation of 1-deaza, 3-deaza, and bridge-elongated analogs of N-10-propargyl-5,8-dideazafolic acid. J Med Chem 34:2746–2754

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Lohn M, Plettenburg O, Ivashchenko Y, Kannt A, Hofmeister A, Kadereit D, Schaefer M, Linz W, Kohlmann M, Herbert JM, Janiak P, O'Connor SE, Ruetten H (2009) Pharmacological characterization of SAR407899, a novel rho-kinase inhibitor. Hypertension 54:676–683

Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K (1994) Selective-inhibition of inducible cyclooxygenase-2 in vivo is antiinflammatory and nonulcerogenic. Proc Natl Acad Sci U S A. 91:3228–3232

Matsui T, Sugiura T, Nakai H, Iguchi S, Shigeoka S, Takada H, Odagaki Y, Nagao Y, Ushio Y, Ohmoto K, Iwamura H, Yamazaki S, Arai Y, Kawamura M (1992) Novel 5-HT3 antagonists—isoquinolinones and 3-aryl-2-pyridones. J Med Chem 35:3307–3319

Mokrosz MJ, Mokrosz JL, Duszynska B, DerenWesolek A, Klodzinska A, Kowalski P, Charakchieva-Minol S, Tatarczyńska E, Kowalska T, Majka Z, Chojnacka-Wójcik E, Misztal S (1997) 5-HT1A and 5-HT2A receptor affinity and functional profile of some N-[3-(4-aryl-1-piperazinyl)propyl] derivatives of indolin-2(1H)-one, quinolin-2(1H)one and isoquinolin-1(2H)-one.30. Structure-activity relationship studies of CNS agents. Pharmazie 52:423–428

Mor M, Rivara S, Pala D, Bedini A, Spadoni G, Tarzia G (2010) Recent advances in the development of melatonin MT1 and MT2 receptor agonists. Exp Opin Ther Pat 20:1059–1077

Ouchi H, Kawata Y, Ono M, Morita Y, Yamamoto Y, Takahata H (2004) Regioselective aromatic substitution of 6,8-dihydroxy-4-ethoxycarbonyl-*2H*-isoquinolin-1-one derivatives using the stille coupling reaction. Heterocycles 62:491–501

Paramashivappa R, Kumar PP, Rao PVS, Rao AS (2003) Design, synthesis and biological evaluation of benzimidazole/benzothiazole and benzoxazole derivatives as cyclooxygenase inhibitors. Bioorg Med Chem Lett 13:657–660

Pettit GR, Meng YH, Herald DL, Graham KAN, Pettit RK, Doubek DL (2003) Isolation and structure of ruprechstyril from *Ruprechtia tangarana*. J Nat Prod 66:1065–1069

Polisson R (1996) Nonsteroidal anti-inflammatory drugs: practical and theoretical considerations in their selection. Am J Med 100:31S–36S

Rajakumar P, Anandhan R (2011) Synthesis and in vitro anti-inflammatory activity of novel glycodendrimers with benzene 1,3,5 carboxamide core and triazole as branching unit. Eur J Med Chem 46:4687–4695

Rajakumar P, Rasheed AMA, Rabia AI, Chamundeeswari D (2006) Synthesis and study of anti-inflammatory activity of some novel cyclophane amides. Bioorg Med Chem Lett 16:6019–6023

Rigby JH, Maharoof USM, Mateo ME (2000) Studies on the narciclasine alkaloids: total synthesis of (+)-narciclasine and (+)-pancratistatin. J Am Chem Soc 122:6624–6628

Shafi S, Alam MM, Mulakayala N, Mulakayala C, Vanaja G, Kalle AM, Pallu R, Alam MS (2012) Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: their anti-inflammatory and anti-nociceptive activities. Eur J Med Chem 49:324–333

Siddiqui RA, English D, Harvey K, Cui Y, Martin MI, Wentland J, Akard L, Jansen J, Thompson J, Garcia JGN (1995) Phorbol ester-induced priming of superoxide generation by phosphatidic acid-stimulated neutrophils and granule-free neutrophil cytoplasts. J Leukoc Biol 58:189–195

Simonsen KB, Juhl K, Steiniger-Brach B, Nielsen SM (2010) Novel NK3 receptor antagonists for the treatment of schizophrenia and other CNS indications. Curr Opin Drug Discov Devel 13:379–388

Snow RJ, Cardozo MG, Morwick TM, Busacca CA, Dong Y, Eckner RJ, Jacober S, Jakes S, Kapadia S, Lukas S, Panzenbeck M, Peet GW, Peterson JD, Prokopowicz AS 3rd, Sellati R, Tolbert RM, Tschantz MA, Moss N (2002) Discovery of 2-phenylaminoimidazo[4,5-h]isoquinolin-9-ones: a new class of inhibitors of lck kinase. J Med Chem 45:3394–3405

Sugimoto A, Shinbatanaka H, Ishikawa M (1995) A new synthetic route to isoquinolin-1(2h)-one derivatives from 3-hydroxyphthalides. Synthesis: 431–434

Tan AS, Berridge MV (2000) Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt, WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. J Immunol Methods 238:59–68

Tonk RK, Bawa S, Chawla G, Deora GS, Kumar S, Rathore V, Mulakayala N, Rajaram A, Kalle AM, Afzal O (2012) Synthesis and pharmacological evaluation of pyrazolo[4,3-c]cinnoline derivatives as potential anti-inflammatory and antibacterial agents. Eur J Med Chem 57:176–184

Tyagi V, Khan S, Giri A, Gauniyal HM, Sridhar B, Chauhan PMS (2012) A ligand-free Pd-catalyzed cascade reaction: an access to the highly diverse isoquinolin-1(2*H*)-one derivatives via isocyanide and Ugi-MCR synthesized amide precursors. Org Lett 14:3126–3129

Viswanadhan VN, Ghose AK, Revankar GR, Robins RK (1989) Atomic physicochemical parameters for 3 dimensional structure directed quantitative structure–activity relationships. 4. Additional parameters for hydrophobic and dispersive interactions and their application for an automated superposition of certain naturally-occurring nucleoside antibiotics. J Chem Inf Comput Sci 29:163–172

Wang F, Liu HX, Fu H, Jiang YY, Zhao YF (2009) An efficient one-pot copper-catalyzed approach to isoquinolin-1(2*H*)-one derivatives. Org Lett 11:2469–2472

Waring MJ, Ben-Hadda T, Kotchevar AT, Ramdani A, Touzani R, Elkadiri S, Hakkou A, Bouakka M, Ellis T (2002) 2,3-bifunctionalized quinoxalines: synthesis, DNA interactions and evaluation of anticancer, anti-tuberculosis and antifungal activity. Molecules 7:641–656

Wu F, Büttner FH, Chen R, Hickey E, Jakes S, Kaplita P, Kashem MA, Kerr S, Kugler S, Paw Z, Prokopowicz A, Shih CK, Snow R, Young E, Cywin CL (2010) Substituted 2*H*-isoquinolin-1-one as potent Rho-Kinase inhibitors. Part 1: hit-to-lead account. Bioorg Med Chem Lett 20:3235–3239

Zhu F, Lin YC (2006) Marinamide, a novel alkaloid and its methyl ester produced by the application of mixed fermentation technique to two mangrove endophytic fungi from the South China Sea. Chin Sci Bull 51:1426–1430